Positive Interference with the Jaffé Reaction by Cephalosporin Antibiotics

Richard R. Swain and Stephen L. Briggs

Cephaloglycin, cephalothin, cephaloridine, cefoxitin, and cephalotetline are cephalosporin antibiotics that react with alkaline picrate solution to give a creatinine-like response. The mechanism of this reaction is not known, and several structurally similar compounds do not react under these same conditions. Because large quantities of a cephalosporin antibiotic may be administered to a patient with limited ability to excrete the drug (as occurs with compromised renal function), the magnitude of this interference can be appreciable. It does not appear possible to avoid the cephalosporin-caused interference by the use of "kinetic" creatinine methods.

The fact that drugs can interfere with clinical chemical testing has been well documented in the recent literature (1-5). Of all drug compounds, antibiotics, especially, might be expected to be troublesome in this regard because they frequently are administered in large amounts. Because the cephalosporins (Figure 1) are widely prescribed antibiotics (6), we chose to evaluate them for interference with commonly performed clinical chemistry tests. Reports have appeared describing interferences by certain of these compounds with urinary protein and urinary glucose (copper-reduction methodology) determinations (7,8). One of these compounds, cephalothin, has also been observed to be a positive interferant in a urine 17-ketosteroid determination (9). In our preliminary investigations of the cephalosporins for interferences, we noted that some of them reacted with alkaline picrate [Jaffé procedure (10)] to give a creatinine-like reaction. Because of the critical importance of the creatinine determination for the assessment of renal function, we decided to study the magnitude and cause of this interference.

Materials and Methods

The automated creatinine procedure used in these studies was performed on the Programachem 1040 analyzer (American Monitor Corp., Indianapolis, Ind. 46288) with use of a reagent containing 1.2 g of picric acid, 8.4 g of NaCl, 2.8 g of NaOH, and 0.5 ml of the surfactant "Brij-35" per liter. In this procedure, 150 μl of sample was added to 4.0 ml of alkaline picrate reagent and allowed to stand for 8 min at ambient temperature, at the end of which time the absorbance was measured at 505 nm. Each sample was individually blanked by subtracting the absorbance of a complete reaction mixture (sample plus reagent) incubated for 12 s from one incubated for 8 min.

Time-course studies were done and absorbance spectra obtained with a Cary 15 spectrophotometer (Varian Associates, Palo Alto, Calif. 94303). Cephalosporin antibiotics were laboratory assay standards. The sources of these compounds were the following—cefamandole, cephaloglycin (e), cephalothin, cephaloridine, cefazolin (cephazolin), cephalaxin (Eli Lilly & Co., Indianapolis, Ind. 46206); cefoxitin (Merck Sharp & Dohme, West Point, Pa. 19486); cephalotetline (Ciba Pharmaceutical Co., Summit, N.J. 07901); cephradine (E. R. Squibb & Sons, Princeton, N.J. 08540); and cephaloridine (Bristol Laboratories, Syracuse, N.Y. 13201). Broad Spectrum Mixture β-lactamase (penicillin amid β-lactamase, EC 3.5.2.6) was obtained from Whatman Biochemicals Ltd., Maidstone, Kent, U.K., and contained 50 units β-lactamase II and 500 units β-lactamase I per vial. The content of one vial was dissolved in 5.0 ml of 10 mmol/liter phosphate buffer, pH 7.4.

Results and Discussion

The magnitude of the creatinine-like response given by certain cephalosporin compounds in the automated creatinine procedure is shown in Table 1. In contrast to the compounds listed there, the following closely related drugs gave negative or very slight responses under the same assay conditions: penicillin G, cefazolin, cephalaxin, cephradine, cefamandole, and cephaloridine. It is noteworthy that on a molar basis the magnitude of the creatinine-like response is much less than that given by creatinine itself (10 mmol/liter gives a response equivalent to 1131 mg of creatinine per liter). However, appreciable concentrations of cephalosporins can appear in blood and urine, because administration of 10 g/day or more of these compounds is not uncommon. Concentrations in blood of 1 g/liter have been achieved for cephalothin in one patient (Lilly Research Laboratories, unpublished data). Cephalothin in this concentration would afford an increase in apparent creatinine concentration of about 19 mg/liter.

Table 2 shows results given by a pooled sample of serum in which sodium cephalothin was dissolved before analysis by use of the Programachem 1040 and standard AutoAnalyzer procedures (Method N-11b; Technicon Instruments Corp., Tarrytown, N.Y. 10591). The purpose of this experiment was to compare the interference produced in a procedure for creatinine as performed directly on serum (Programachem) with that given in a continuous-flow system in which dialysis occurs. It was also of interest to determine whether, in the Programachem procedure, the presence of serum proteins would affect the creatinine-like response given by the cephalosporins. (The studies in Table 1 were performed with the drug dis-
solved in phosphate buffer only.) The results obtained show a greater interference when the Jaffé reaction is performed directly on serum (i.e., without a dialysis step). This observation might be expected, because cephalothin is partly bound to serum proteins (6), and it also would diffuse across the dialysis membrane more slowly than creatinine because of its greater molecular weight. The reaction of cephalothin with alkaline picrate appears not to be affected by serum proteins, because the creatinine-like reactivity seen in the Programachem procedure in Table 2 (protein present) is about the same as that in Table 1 (no protein present) with 1.0 g of cephalothin per liter being equivalent to 18 mg of creatinine per liter.

Because we thought it to be of interest to discern the nature of the creatinine-like reactivity, we attempted to determine which portion of the cephalosporin structure might be responsible for the reaction with alkaline picrate. Apparently, the cephem nucleus (composed of the dihydrothiazine and \(\beta\)-lactam rings) alone is not responsible for the reaction, because this structure is common to all the cephalosporins whereas only those compounds listed in Table 1 (of those we tested) give the creatinine-like response. We did decide, however, to treat certain of the reacting cephalosporins with a \(\beta\)-lactamase preparation to determine the effect of disruption of the \(\beta\)-lactam ring on the reactivity with picrate. The results (Table 3) indicate that complete or partial hydrolysis of this structure does abolish or lessen the creatinine-like reaction. Apparently, hydrolysis of the \(\beta\)-lactam ring of cephaloglycine is incomplete under these conditions. Note that neither cefoxitin, which is resistant to \(\beta\)-lactamase, nor creatinine lose any of their reactivity. Therefore, it would appear that the \(\beta\)-lactam ring itself is not responsible for the creatinine-like reaction because all cephalosporins contain this structure, yet not all are reactive. On the other hand, an intact ring is somehow necessary for this reactivity because hydrolysis of this structure results in loss of ability to give the Jaffé reaction.

We also observed that several of the compounds that we tested and found reactive contained a thiophene side chain. To investigate the possibility that this structure might be reactive in those compounds that contain it, we tested 10 mmol/liter solutions of thiophene, thiophene carboxylic acid, and thiophene carbonitrile under the same conditions as for the studies in Table 1, but saw no reaction.

Studies on the stability of the cephalosporins in basic solutions have been done (11), and we see no apparent correlation between lability in basic medium and the creatinine-like reaction. The nature of the reaction with alkaline picrate is unknown, but presumably a species such as that described by Butler (12) may be formed.

Figure 2 shows the time courses of the reactions with alkaline picrate for positively reacting compounds and for creatinine itself. Concentrations were chosen so as to provide about the same final absorbances after 10 min. The time course is somewhat different for each compound, but in every case the cephalosporin reacts more rapidly under these conditions than does creatinine itself. In a separate series of experiments, we incubated these compounds in alkali alone for various times.

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**Table 1. Creatinine-Like Reaction Given by Certain Cephalosporin Antibiotics**

<table>
<thead>
<tr>
<th>Cephalosporin</th>
<th>Conc*</th>
<th>Apparent creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephaloglycin</td>
<td>10</td>
<td>38</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>10</td>
<td>79</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Cephacetrile</td>
<td>10</td>
<td>66</td>
</tr>
</tbody>
</table>

* Drugs were dissolved in 10 mmol/liter phosphate buffer, pH 7.4.

**Table 2. Creatinine-Like Response of Cephalothin in Programachem and AutoAnalyzer I Procedures**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Creatine concn., mg/liter</th>
<th>Prog. 1040</th>
<th>AA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum pool</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Serum pool (cph. 0.6 g/liter)</td>
<td>19</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Serum pool (cph. 1.0 g/liter)</td>
<td>25</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Creatinine-Like Response after Treatment with \(\beta\)-Lactamase**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc, g/liter</th>
<th>Apparent creatinine, mg/liter</th>
<th>Initial</th>
<th>After (\beta)-lactamase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalothin</td>
<td>2.2</td>
<td>39</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cephaloglycin</td>
<td>2.8</td>
<td>46</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>3.3</td>
<td>66</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.045</td>
<td>44</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

Creatinine assay was performed on the Programachem 1040. Drugs were dissolved in 10 mmol/liter phosphate buffer, pH 7.4. \(\beta\)-Lactamase preparation (0.5 ml) was added to 5.0 ml of drug solution. Incubation was at 37 °C for 30 min.

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**Fig. 1. Structures of cephalosporin antibiotics**

The cephem nucleus, composed of the six-member sulfur-containing ring (dihydrothiazine ring) and the \(\beta\)-lactam ring, is common to all of these compounds.
Creatinine was spontaneously produced from incubation. However, this was shown to be identical with the Cephalotrin method. The results presented here indicate that certain cephalosporins can give a creatinine-like response with alkaline picrate by an as-yet-unknown mechanism. The magnitude of this interference can vary from negligible to appreciable, depending on such factors as amount of antibiotic administered and the renal function of the patient. Interference is greater in "direct" creatinine methods than in those procedures that include a dialysis step. Although several "kinetic" creatinine methods have been devised, in which the attempt is made to achieve specificity by isolating the reaction due to creatinine from that caused by faster- or slower-reacting components (14, 15), the studies shown in Figure 3 indicate that the cephalosporin interference cannot be avoided in this manner. Only the development of a completely creatinine-specific methodology will avoid this type of interference entirely. The fact that interferences of this nature exist is a strong argument for the use of methods specific for the substance of interest.

References


Ed. note: Interference by cephalothin was noted a little earlier by Watkins et al. [Microchem. J. 21, 370 (1976)].